# Development of the Muscular Network of the Midgut in the Larval Stages of the Mosquito, *Aedes Aegypti* Linnaeus[1]

JAMES F. O'BRIEN[2]
BIOLOGICAL LABORATORIES, FORDHAM UNIVERSITY, BRONX, NEW YORK 10458

**Abstract:** Attempts to find an organized muscle layer surrounding the midgut of the early instar larval *Aedes aegypti* mosquito have not been successful. But, by studying whole mounts of larval midguts of various stages, muscle cells were found to be present from the earliest stages of larval life. The early, first-instar muscle layer consists of two pairs of longitudinally arranged rows of muscle cells connected by two circular bands of muscle cells. Beginning at about the second instar, these cells begin to undergo mitotic divisions, eventually giving rise to a muscular network, completely encircling the larval midgut. The network present in the prepupal midgut apparently remains during metamorphosis to become the muscular coat of the adult midgut.

Christophers (1960) has summarized most of the biological findings concerned with almost every aspect of the mosquito *Aedes aegypti* Linnaeus. But his description of the histological findings concerning the presence of muscles in the larval midgut revealed that little work had been done in this area and there was still doubt as to the presence of a muscular coat in the earlier larval stages. Samtleben (1929) reported the presence of scattered muscle fibers on the midgut in the early instars. Christophers was unable to detect muscle fibers in the early instars but has described the musculature he observed in the fourth-instar larval midgut. Because of this doubt and conflicting reports, the study of the cytological development of the *A. aegypti* midgut from the early hours of larval life, through pupation and into the adult, was undertaken. This investigation is concerned mainly with the larval midgut development, but an attempt is made to correlate larval structures with those found in the imaginal midgut.

## MATERIALS AND METHODS

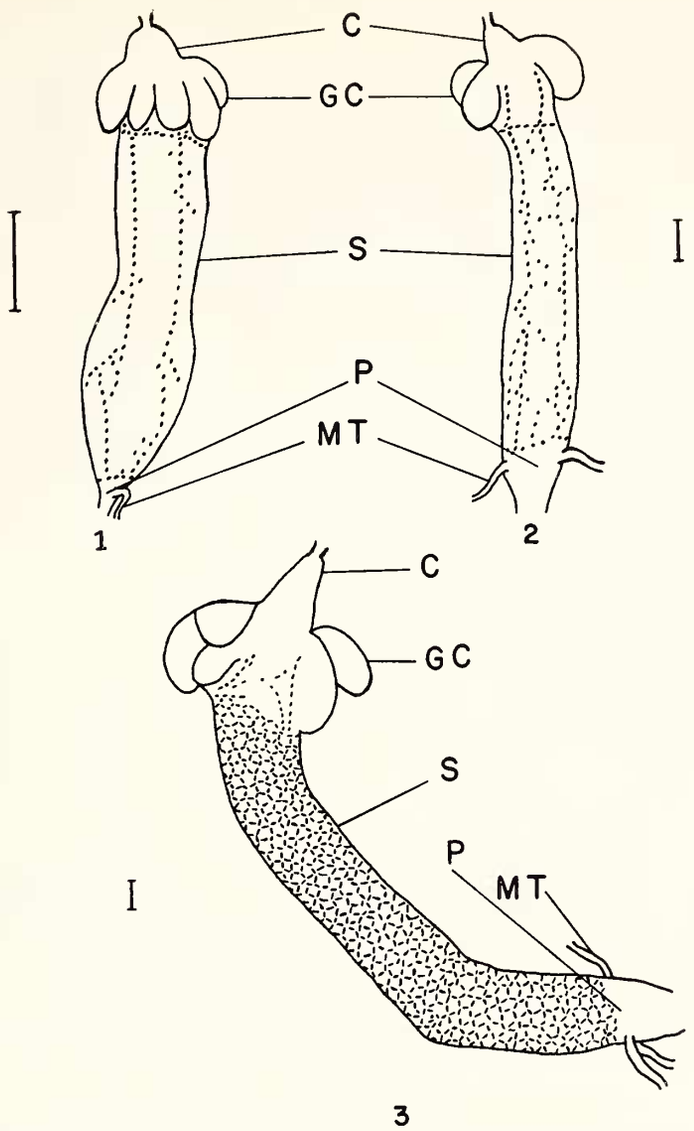
The eggs used to obtain larvae were collected from the *A. aegypti* colony maintained in this laboratory during the course of the research. This colony was originally developed from eggs obtained from Rutgers University. The larvae were reared in tap water and fed a watery suspension of brewers' yeast.

Beginning about 6 hours after hatching and continuing into the pupal and freshly emerged adult stages, a series of whole mounts of midguts were prepared, stained by the Feulgen reaction and counterstained with Orange G. The whole

---

[1] A portion of the author's dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Fordham University. The author wishes to acknowledge the assistance and encouragement given him during this study by Prof. C. A. Berger, S.J., of the Fordham Biological Laboratory. This work was supported in part by an Educational Assistance Grant from the Arthur J. Schmitt Foundation.

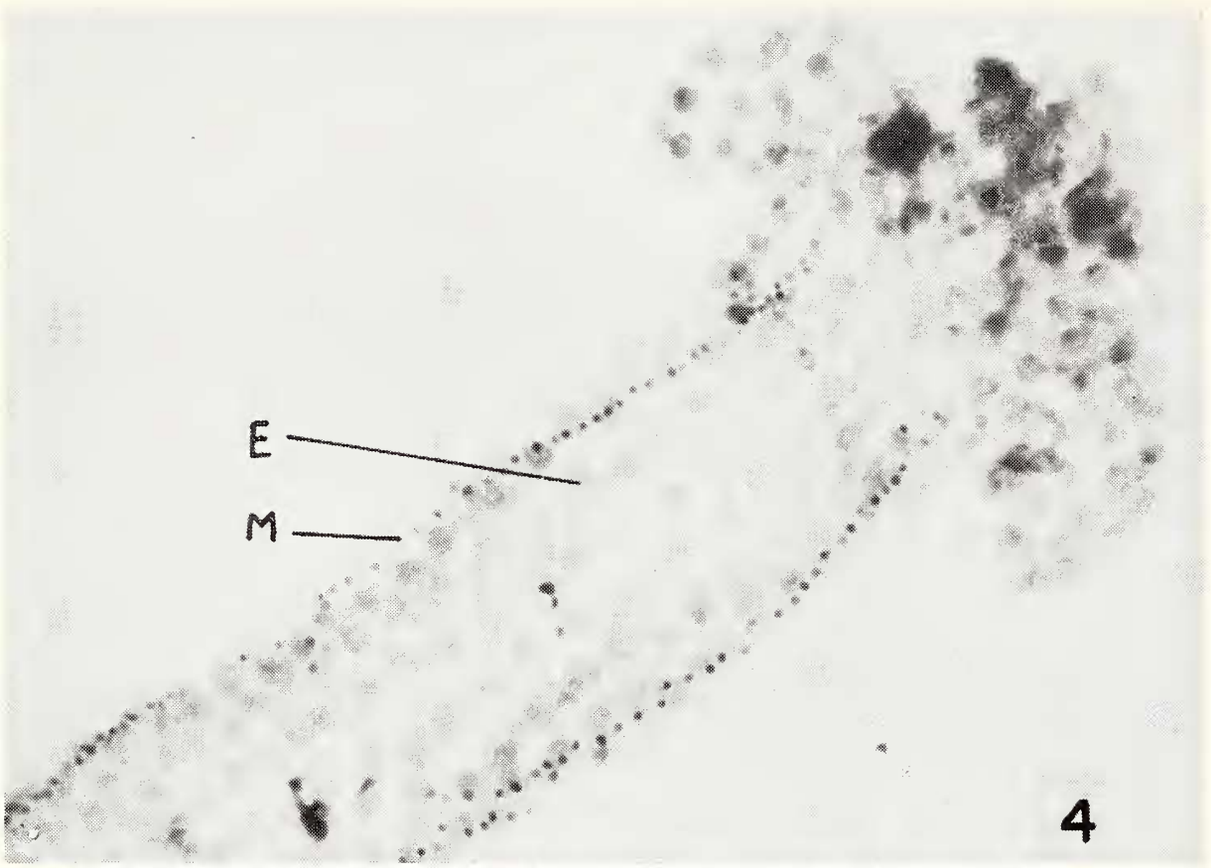
[2] Present address: Regis College, Willowdale, Ontario, Canada.




Figs. 1–3. Diagrammatic representation of the formation of the muscular network beginning with the longitudinal and circular rows of muscle cells in the first instar and terminating in the fully formed network in the prepupa. Scale lines = 0.1 mm. C—Cardiac region; GC—Gastric ceca; MT—Malpighian tubule; P—Pyloric region; S—Stomach.

Fig. 1—9-hour larva; Fig. 2—40-hour larva; Fig. 3—166-hour larva (prepupa).

mounts of this series represent intervals of from 1 to 4 hours of development. Dissections were performed in *Drosophila* saline (Demerec and Kaufmann, 1961) and Formalin–Acetic Acid–Alcohol (FAA) was used as the fixative. Since the larval midgut is small and delicate, the dissection, the fixation, and the staining were performed on depression slides. After the dissection, the midgut remained on the depression slide until staining and dehydration were completed. Solutions were added to the depression slide with droppers and removed with rolled pieces of filter paper. Hydrolysis in N/HCl was carried out for 7–9 minutes in a paraffin oven at 52–55° C. After staining and dehydration through alcohol and xylol, the midgut was flooded from the depression slide onto a clean microscope slide and mounted in permount. Such a procedure helped to eliminate loss or damage to the tissue during processing. The whole mounts were



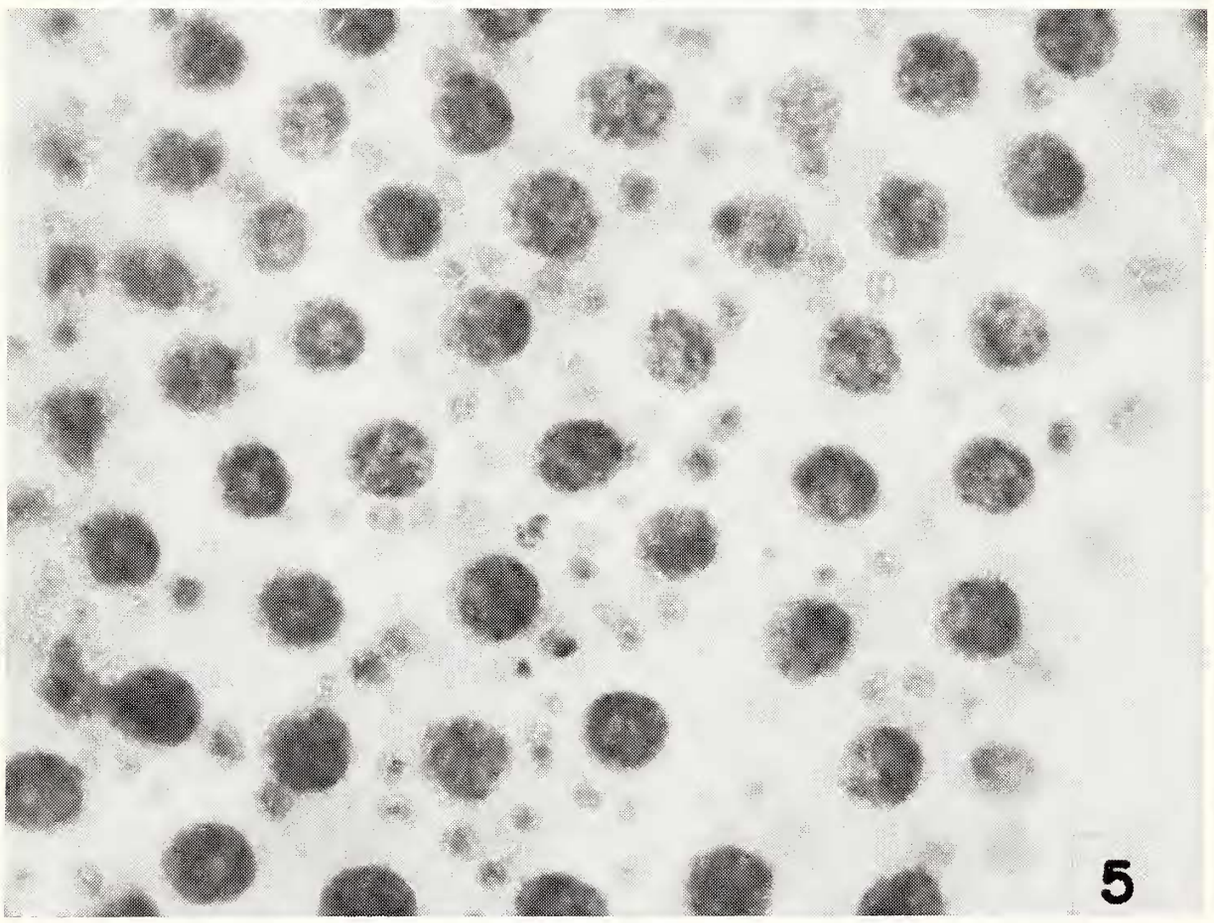




FIG. 4. Photomicrograph of anterior portion of 24-hour larval midgut showing the rows of muscle cells overlying the epithelial cells. E—Epithelial cell nucleus; M—Muscle cell nucleus. × 280.

FIG. 5. Photomicrograph of portion of stomach of 40-hour larva showing the beginnings of the formation of the muscle network. Large nuclei are epithelial cell nuclei. × 570.

then examined by light microscopy. All photomicrographs were made with a Miranda-F 35 mm camera.

## RESULTS

This study reveals that from the beginning of larval life, muscles are present in the midgut. The first indication of the presence of muscles in the early stages was the peristalsis observed in the freshly dissected midguts.

In the whole mounts, the first-instar midgut muscles appear as four longitudinal rows of deeply stained cells connected by at least two rows of similar cells which encircle the midgut (Figs. 1 and 4). One of the circular rows is located just below the level of the gastric ceca and the second circular row is found just above the pyloric region. Examination reveals that the longitudinal rows of cells continue into the area beneath the pouches of the gastric ceca.

The arrangement of muscle cells in the early first-instar midgut remains essentially unchanged until about the 30th hour, or the beginning of the second instar. At this time, numerous mitotic divisions of these cells appear and by the 40th hour, the beginnings of a muscular network can be discerned (Figs. 2 and 5). From this time on, as the midgut grows in size, muscle cell divisions continue until a dense pattern of muscle fibers forms around the midgut (Figs. 3 and 6).

Divisions of muscle cells are normal mitotic divisions showing the somatic pairing of homologous chromosomes characteristic of dipteran cells (Fig. 7). In forming the muscle network, the positions taken by the muscle cells form a pattern which more or less outlines the basal edges of the large epithelial cells of the midgut (Figs. 7 and 8).

No evidence was found to indicate a double layer of muscle cells. The muscular network in the midgut of *A. aegypti* is comprised of a single layer of muscle cells. There is no evidence of a network around the gastric ceca. In only a few of the larvae dissected, an occasional muscle strand was seen attached to a pouch of the ceca.

The muscular network is completed by the late fourth instar or prepupa and survives the changes involved in the formation of the imaginal gut during the pupal stage to become the muscular network around the imaginal midgut.

## DISCUSSION

The results of this study show that while a complete network of muscle fibers is not present in the *Aedes* midgut until the later larval instars, the peristalsis observed in freshly dissected midguts of all stages confirms muscular activity from the beginning of larval feeding. Since the muscular layer is delicate and easily torn from the gut during dissection or processing, perhaps the inability of earlier investigators to find any more than a few strands of muscle fibers might have been due to the accidental removal of the muscle coat in dissection or staining.

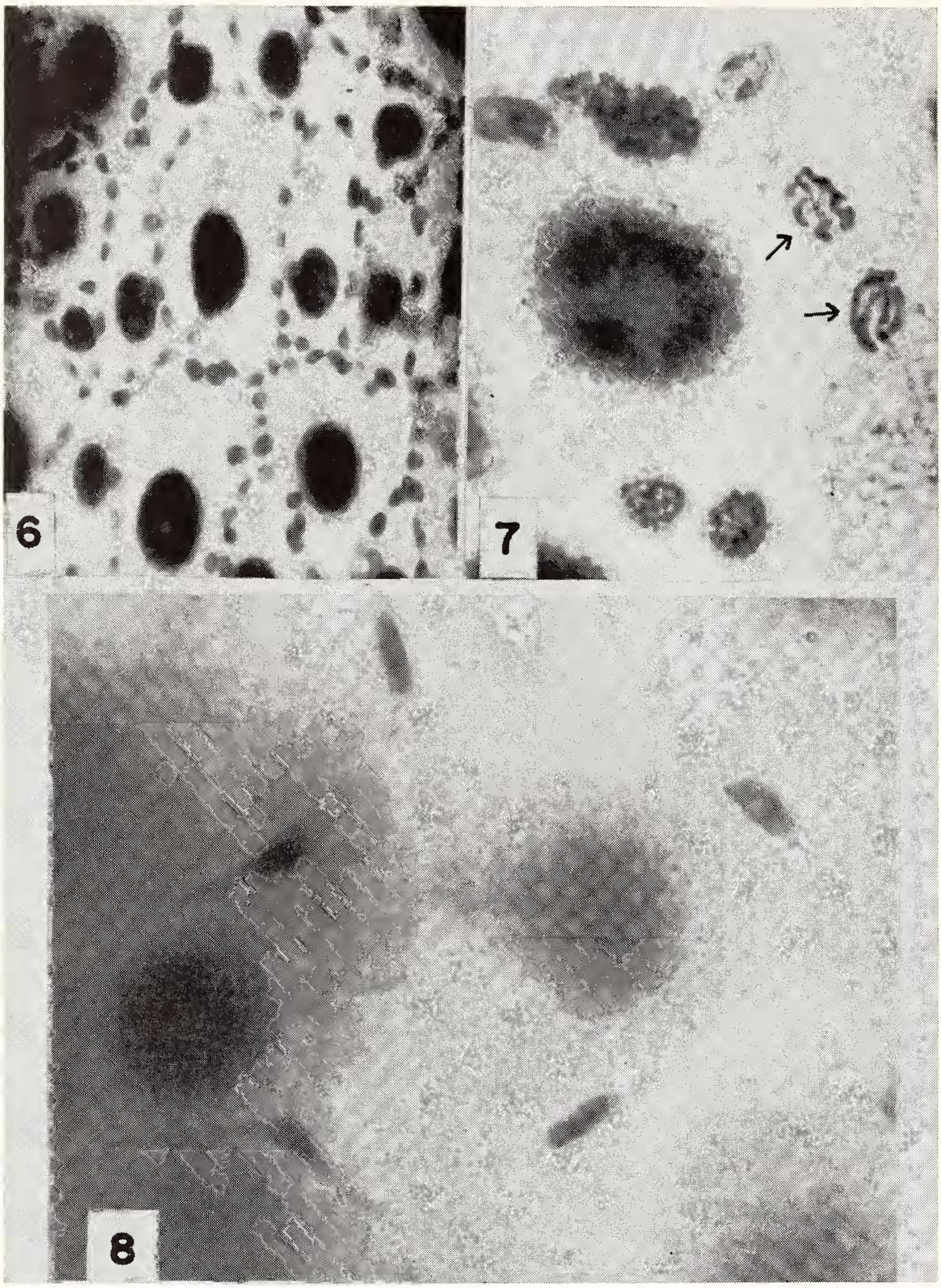



FIG. 6. Photomicrograph of portion of stomach of 166-hour larva showing the pattern of the completely formed muscle network. Large nuclei are epithelial cell nuclei. × 570.

FIG. 7. Photomicrograph of portion of stomach of 72-hour larva. Arrows indicate two muscle cells undergoing mitotic division. Large nuclei are epithelial cell nuclei. × 1,290.

FIG. 8. Photomicrograph of portion of stomach of 144-hour larva showing the positions taken by the muscles in the formation of the network. × 1,290.

It seems strange that the arrangement of the muscle cells in the early larval midgut, as described above, was not found by Christophers until the fourth-larval instar. Christophers' findings appear to indicate a retarded condition in the formation of the muscular system of the midgut.

The *Culex* larval midgut musculature, as reported by Berger (1938), consists of separate longitudinal and circular layers of muscle fibers, while the network present in the *Aedes* larva consists of only a single layer of muscle cells arranged to form a mesh covering the stomach area of the midgut. In the fourth-larval instar, the bases of the enlarged epithelial cells bulge outward through the interstices of this muscular reticulum. Peristalsis produced by such a network is probably effected by each muscle contracting along its long axis, thereby producing both circular and longitudinal constriction of the midgut.

The failure of a muscular network to form around the gastric ceca of the larval midgut is logical. The ceca break down during the pupal stage and are not present in the imaginal midgut. Since there is nothing in the adult midgut to correspond to the pouches of the larval gastric ceca, any muscular network formed around these diverticula in the larval stages would be useless in the adult gut. The tubular midgut of the adult replaces the larval midgut by fitting into the muscular network that developed around the midgut during the larval stages.

SUMMARY

Muscle cells are present in the midgut of *A. aegypti* from the earliest stages of larval life.

The initial arrangement of muscles consists of two pairs of longitudinal rows of muscle cells joined by at least two rows of similar cells encircling the midgut.

At about the 30th hour of larval life, mitotic divisions of muscle cells increase in number and a network of muscle cells begins to form.

By the fourth instar the larval stomach is enclosed by a muscular network that is one layer thick. This network will become the muscle coat of the imaginal midgut.

No such network forms in the region of the gastric ceca.

## Literature Cited

Berger, C. A. 1938. Multiplication and reduction of somatic chromosome groups as a regular developmental process in the mosquito, *Culex pipiens*. Pub. 496. Carnegie Institution of Washington.

Christophers, S. R. 1960. *Aedes aegypti* (L.). Cambridge Univ. Press.

Demerec, M., and B. P. Kaufmann. 1961. *Drosophila Guide*. 7th Ed. Carnegie Institution of Washington.

Samtleben, B. 1929. Zur Kenntnis der Histologie und Metamorphose des Mitteldarms der Stechmückenlarven. Zool. Anz. **81:** 97–109.

Received for Publication August 11, 1965